View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

inhibitors has been considerably hampered. Diverse agents such as La3+, Gd3+, 2-APB and imidazole derivatives inhibit CRAC currents, however, in a non-selective manner limiting their usefulness as CRAC blockers. The two key molecular components of the CRAC channels: STIM1 and Orai1 which were identified 5-6 years ago, represent promising targets for the elucidation of novel and selective CRAC blockers. Here, we present two novel inhibitors, GSK-7975A and GSK-5503A, which fully inhibited Orai1 and Orai3 currents with a substantially slower rate of onset than La3+. Inhibition of Orai currents occurred with an IC50 of ~ 4μM and exhibited limited reversibility upon washout. Blockage of currents through the less Ca2+-selective pore mutant Orai1 E106D (or 2-APB stimulated Orai3) was significantly reduced suggesting that the selectivity filter is a direct or allosteric target of these GSK CRAC channel blocking compounds. Furthermore, FRET experiments indicated that neither STIM1-STIM1 oligomerization nor STIM1-Orai1 coupling was affected by these compounds. The endogenous CRAC current of RBL cells was also inhibited by these compounds whereas amongst other Ca2+-selective channels, L-type Ca2+ currents exhibited only slight inhibition and TRPV6 currents were fully blocked. In summary, the elucidation of these novel CRAC current blockers represents an important step forward in the identification of CRAC channel-selective drug molecules. (supported by the Austrian Science Fund (FWF): T466 and P22565)

1602-Pos Board B372

Store-Operated Calcium Signaling in the Airway Epithelium- Functions and Molecular Mechanisms

Amit Jairaman, Murali Prakriya.
Northwestern University, Chicago, IL, USA.

Ca^{2+} is a ubiquitous second messenger that regulates a variety of essential cellular functions such as gene transcription, exocytosis and cell motility. In many cell types, store-operated Ca^{2+} release-activated Ca^{2+} (CRAC) channels have emerged as a major mechanism of generating Ca^{2+} signals. In the conducting airways of the lung, airway epithelial cells (AECs) lining the respiratory tract play a central role in orchestrating both innate and adaptive immune responses. AECs shape the nature of the immune response in the airway by coordinating the recruitment and activation of immune cells through the release of a host of inflammatory mediators including various cytokines and chemokines. While a great deal has been learnt about the various downstream inflammatory mediators released by AECs, the upstream signaling pathways that lead to the generation of these mediators remain poorly understood. While Ca^{2+} fluxes have been shown to play an important role in initiating gene transcription and mediating cytokine release in many immune cell types, the nature and sources of Ca^{2+} entry that initiate the generation of cytokines in AECs remain poorly defined. Our initial results show that CRAC channels are a major route of Ca^{2+} entry in the airway epithelium. Furthermore, CRAC channel activation stimulates robust gene expression in AECs leading to the generation of a few key inflammatory modulators in the respiratory tract. We show that certain allergens and purinergic agonists which are known to play an important role in the airway inflammatory response associated with asthma stimulate sustained Ca^{2+} influx in AECs by activating CRAC channels. Based on these findings, we propose that CRAC channels constitute a key checkpoint in mediating inflammatory responses in the respiratory tract by potentially modulating the responses of the airway epithelium to various pathogens and allergens.

1603-Pos Board B373

Overexpression of Orai1 and STIM1 can Change Some Properties of Endogenous SOCE

Tomasz Gwozdz, Joanna Dutko-Gwozdz, Nadine Aziz,
Victoria M. Bolotina.
Boston University School of Medicine, Boston, MA, USA.

Orai1 and STIM1 have been identified as main components of store-operated Ca^{2+} entry (SOCE), and their molecular interactions have been extensively studied in heterologous overexpression systems. Here we used molecular and imaging techniques to assess if overexpression of Orai1 and STIM1 may alter some physiological properties of endogenous SOCE pathway, and if there may be a crosstalk between expression levels of different components of SOCE. We found that in contrast to significant inhibition of endogenous SOCE caused by functional or molecular knock down of Ca^{2+}-independent phospholipase A_2 (PLA2G6) in naïve HEK293 cells, the loss of SOCE was significantly attenuated in cells in which Orai1 or STIM1 were overexpressed. Similarly, overexpression of Orai1 or STIM1 prevented the effects of depletion of plasma membrane cholesterol on SOCE: β-methyl cyclodextrin caused significant inhibition of SOCE in naïve, but not Orai1/STIM1 overexpressing cells. Further, we found crosstalk between expression levels of endogenous Orai1 and the plasma membrane variant of PLA2G6, but not STIM1. siRNA-induced knock down of Orai1 caused up to 6 fold increase in expression level of PLA2G6, but caused no change in STIM1 expression in HEK293 cells. Reciprocally, siRNA-induced down-regulation of PLA2G6 caused about 2 fold increase in endogenous Orai1 expression. Knock down of STIM1 had no effect on expression levels of either Orai1 or PLA2G6. Similar results were obtained in human aortic smooth muscle cells. These data suggest that there is an interdependence of expression levels of Orai1 and PLA2G6, and overexpression of either Orai1 or STIM1 may significantly influence the important physiological properties of SOCE. Further studies of endogenous SOCE mechanism are needed to determine the full spectrum of its molecular and functional components that may be required for effective signal transduction in naïve cells.

1604-Pos Board B374

Interactions Between Secretory Pathway Ca2+-ATPase 2 with TRP and Orai Channels Mediate Store Independent Ca2+ Entry in Lactation

Brandie M. Cross[1], Mingye Feng[1], Rajini Rao[1], Tim Reinhardt[2].
[1]Johns Hopkins University, School of Medicine, Baltimore, MD, USA,
[2]USDA-Agricultural Research Service, National Animal Disease Center, Ames, IA, USA.

Secretory pathway Ca2+ ATPases (SPCAs) are important in sequestering Ca2+ and Mn2+ from the cytoplasm into the Golgi and post-Golgi vesicles for important post-translational modifications to a multitude of enzymes. The two isoforms, SPCA1 and SPCA2 share high homology but have distinct localization and distribution. Whereas SPCA1 is a ubiquitous and essential protein with conventional Golgi localization, SPCA2 is expressed in highly secretory or absorptive epithelia where it can traffic to the plasma membrane. We showed that SPCA2 interacts with Orai1 to activate store-independent Ca2+ entry in breast cancer (Feng, et al, 2010). However, the normal physiological explanation for these interactions remains to be elucidated. Clinical studies have suggested a possible preventive role for lactation with respect to breast cancer (Neubauer, et al, 1994). In this study, we examine the interaction of SPCA2 with Orai1 and TRP channels throughout lactation. We show that: i) SPCA2 interacts with Orai1 and TRP channels, ii) these interactions are involved in the early stages of lactation and iii) these interactions may play a role in the sequestration of Ca2+ from the blood (2mM Ca2+) to the milk (40-80mM Ca2+).

1605-Pos Board B375

T Cell Activation Causes Little Upregulation of *Orai* Gene Expression and Reduction in Functional CRAC Channel Density

Pratima Thakur, **Alla F. Fomina**.
Univ. California, Davis, Davis, CA, USA.

Ca^{2+} release-activated Ca^{2+} (CRAC) channel-mediated Ca^{2+} entry regulates multiple T lymphocyte functions. T cells activated by crosslinking of T cell receptors display enhanced Ca^{2+} signaling compared with resting T cells; this can be caused by activation-induced upregulation of CRAC channel expression. However, studies of the expression of *Orai* and *Stim* family genes encoding CRAC channel structural elements and regulatory proteins, respectively, produced controversial results. Using quantitative RT-PCR assay we re-examined *Orai* and *Stim* gene expression relative to the stably expressed *B2M* and *RPL13a* reference genes in resting, activated, and Jurkat T cells. Relative levels of *Orai1* transcripts encoding the human T cell CRAC channel pore-forming subunit were not significantly different between primary resting T cells and 5-day activated T cells. The relative amount of all *Orai* transcripts (*Orai1*, *Orai2,* and *Orai3*) was significantly (2-fold) higher in 5-day activated T cells than that in resting T cells. *Orai1* and total *Orai* transcript levels were significantly higher in Jurkat T cells than those in resting T cells. *Stim* expression did not vary significantly among cell types. Maximal whole-cell CRAC current amplitudes were 1.4- and 2.3-fold higher in activated and Jurkat T cells, respectively, than in resting T cells. However, due to the small size of resting T cells, the surface CRAC channel densities were 2.5- and 1.6-fold lower in activated and Jurkat T cells, respectively, than in resting T cells. Analysis of the predicted rates of cytosolic Ca^{2+} elevation evoked by Ca^{2+} entry via CRAC channels calculated using the average CRAC current amplitude and cell volume values revealed that activation-induced upregulation of CRAC channel expression cannot account for the enhanced store-operated Ca^{2+} entry in activated T cells compared with resting T cells.